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REVIEW

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Biomarkers in headaches as a potential solution to simplify differential diagnosis of primary headache disorders: a systematic review

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Abstract

Background According to the International Classification of Headache Disorders, 3rd edition (ICHD-3), headache disorders can be divided into two main groups: primary, which are not caused by any other disease, and secondary, which are symptomatic of underlying disease. Differentiating between both groups is crucial for the patient's prognosis. The diagnosis of primary headache disorders relies solely on official clinical criteria, with no additional diagnostic tools available. Therefore, they usually remain underdiagnosed, decreasing the patient's quality of life.

Methods This systematic review aimed to analyse the available literature on the topic of biomarkers in the differentiation between different types of headaches. To be included, a primary study had to cover the abovementioned topic. Studies comparing one type of headache to healthy controls were excluded since the review focused on differential diagnosis. Articles to be considered had to describe original research and be written in English or Polish. No publication year limits were applied. A selection process was performed between October 19th, 2024, and January 1st, 2025, through six databases (PubMed, Embase, Scopus, Cochrane, Web of Science, Medline Ultimate), according to the PRISMA 2020. The risk of bias was assessed accordingly using the Prediction Model Risk of Bias Assessment Tool (PROBAST), and data synthesis was performed narratively. The review was registered in PROSPERO.

Findings The findings from 21 included studies (with a wide range of publication years between 1990 and 2023) demonstrated several biomarkers, mainly comparing migraine to other primary headaches, tension-type headaches and cluster headaches, and some secondary headaches: medication-overuse headaches and post-traumatic headaches. The main types of biomarkers were blood biomarkers and imaging biomarkers. Among the former, molecules such as magnesium and calcitonin gene-related peptide (CGRP) or inflammatory markers could be found. The latter group focused mainly on assessing volumes or functional connections in brain magnetic resonance imaging and seem to have a significant impact in the nearest future. Saliva analyses were covered only by two research groups, showing the putative role of magnesium and CGRP. Similarly, two research groups described evoked potentials' value only in the paediatric population.

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Conclusions There is a clear gap in the literature regarding biomarkers for the differential diagnosis of headaches. However, an analysis of the most recent studies suggests that imaging biomarkers are the most promising group since they have gained the most attention in the past few years. Finding high-value biomarkers can simplify differential diagnosis of headaches, especially when clinical presentation is atypical. Nevertheless, more research on biomarkers of all types is highly needed.

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Keywords Biomarker, Cluster headaches, Differential diagnosis, Migraine, Primary headache disorders, Tension-type headaches, Trigeminal autonomic cephalalgias

Background

Headache disorders and how to diagnose them

Primary headache disorders are conditions not caused by any other underlying disorder or process. Conversely, secondary headaches are manifestations of other pathologies due to their causal connection [1]. In other words, primary headaches are not symptoms but independent diseases [2]. The International Classification of Headache Disorders, 3rd edition (ICHD-3), in the first part, distinguishes four main groups of primary headaches, which stay as follows: (i) migraine, (ii) tension-type headache (TTH), (iii) trigeminal autonomic cephalalgias, with cluster headache (CH) being the most common one, and (iv) other primary headache disorders [3]. The second part covers secondary headaches and is divided by the type of causal pathology or disease [3]. Each type of headache in ICHD-3 can be diagnosed by fulfilling specific criteria that describe characteristics of likely any kind of headache a clinician may face daily [3].

As mentioned, primary headaches are diagnosed based on official clinical criteria. Despite multiple studies focusing on identifying headache biomarkers, no other validated diagnostic methods exist [4]. Secondary headaches, although a minority of acute headaches met in an emergency department, may be life-threatening and demand fast diagnosis [5]. Therefore, proper diagnosis of headaches relying mainly on the clinical history and thorough physical and neurological examination is crucial [5]. The common approach is based on "red flags" and "green flags" of headaches, which suggest whether, respectively, the headache needs immediate care (typically secondary headaches) or can be treated ambulatory (typically primary headaches) [6]. Additional tests may be applied if accurate; however, they are not necessary in every case [7].

A problem of undiagnosed headaches

The importance of fast diagnosis of life-threatening causes of secondary headaches has gained more attention in recent years [8, 9]. Nevertheless, the issue of underdiagnosis of primary headaches should also be considered a severe problem [10] since the lifetime prevalence of primary headaches is extremely high, with migraine incidence ranging from 17 to 33% and 8–22% in females and males, respectively, and TTH prevalence reaching even 90% [1]. Despite the challenge of making the correct diagnosis, mainly when headache features intermesh between different types, there is an apparent gap in biomarker research, which could potentially facilitate differential diagnosis in atypical cases. When a patient presents with all classic characteristics, the diagnosis process is usually not demanding; however, when not all criteria are met and a definite diagnosis can't be made, the additional test resolving this problem would be undoubtedly beneficial.

Migraine remains constantly underdiagnosed and undertreated [11], causing headache-related disability mainly among young, professionally active women, being not only an individual but also an economic problem [12]. According to the Global Burden of Disease Study 2019, migraine is the second cause of years lived with disability globally and the first among females aged 15–49 [13]. Similarly, despite its usually disabling character, the progress in the management of TTH remains little [14]. Moreover, ICHD-3 distinguishes positions such as probable migraine and TTH [3], and differentiation between these probable diagnoses is often confusing for clinicians [15]. Finally, a proper diagnosis is undoubtedly the key to effectively treating headaches, which is a subject of constant development [16, 17].

Possibilities among headache diagnosis

Although ICHD-3 is a high-value classification that is constantly being improved [3, 18, 19], and at least in theory, diagnosing primary headaches shouldn't face difficulties, the existing problem can't be denied. Recently, diverse biomarkers have arisen as potential opportunities to facilitate headache diagnosis [20–22]. The most studied primary headache in terms of biomarkers is migraine [20], and among the most studied biomarkers are such molecules as calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating peptide-38 (PACAP-38), and inflammation markers, like interleukins [23, 24]. Furthermore, cerebrospinal fluid (CSF) and neuroimaging biomarkers are possibilities other than blood examination [25, 26]. However, in most cases, biomarker

studies compare one specific type of primary headache disorder to healthy individuals and not differential diagnosis between headache types, which would definitely enhance clinical decision-making.

Therefore, this systematic review aimed to cover this insufficiently explored topic by focusing on biomarkers distinguishing headache types. The objective was to indicate biomarkers that could be implemented to facilitate the diagnostic process. We hypothesised that there are diverse biomarkers that could be valuable in the process of headache type differentiation. To our knowledge, this is the first work summarizing biomarkers in the differential diagnosis of primary headaches.

Methodology

The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA 2020) [27]. The electronic search was performed through six databases: PubMed Database, Embase Database, Scopus Database, Cochrane Database, Web of Science Database, and Medline Ultimate Database on October 19th, 2024, by applying the same search strategy for each database: (biomarker) AND (migraine OR headache) AND ('differential diagnosis' OR differentiation). The search of the Embase database followed the same general design, taking into account the specific syntactic needs of the search engine. The specific terms were searched in each database in all fields, and no limitations to manuscript parts such as abstract or title were applied in this area. The Endnote Program 21.0.1 (licensed by the Medical University of Warsaw) was used for the screening process.

The systematic review was registered in PROSPERO, the International Prospective Register of Systematic Reviews (ID: CRD42024603632), and the protocol was followed. Due to the nature of the biomarker research, a meta-analysis was not conducted. The included studies assessed different molecules and methods, and if any of them focused on the same biomarker type, the thresholds used varied. Additionally, the laboratory methodology was not similar for all the studies.

Inclusion and exclusion criteria

The inclusion and exclusion criteria were applied to indicate all appropriate studies. We included articles in which researchers analysed biomarkers in the differential diagnosis of headaches. More precisely, according to the PICOS model, we screened databases for a population (P, population) of headache patients with a diagnostic test performed (I, intervention) to distinguish one type of headache from the other (C, comparison), looking for biomarkers that can be effective in the differential diagnosis of headache types (O, outcome). Studies including patients of all ages were considered. Original studies, such as randomized control trials, non-randomized trials, and observational (cohort, case-control, and cross-sectional) studies, were allowed (S, study design). The information about diagnosing with ICHD criteria was not obligatory; however, it had to be stated that diagnosis of headache was provided by either a medical professional specialising in headache disorders or a neurologist. No date restrictions have been applied; thus, studies of all years have been taken into consideration.

On the contrary, comparing only one type of headache to healthy controls was an exclusion criterion, since the main objective of the review was to explore biomarkers useful in headaches differentiation when the exact diagnosis is unclear. Research on topics other than headaches differentiation was naturally rejected. Unpublished studies (including trial protocols and conference abstracts), case reports, case series, commentaries, and editorials were excluded. Finally, studies written in languages other than English or Polish were not considered; however, no such articles that otherwise met inclusion criteria were found through all six databases.

Selection process

Two independent authors (O.G. and W.Ł) performed the screening. Any discrepancies were then additionally analysed and re-assessed. If two authors could not resolve differences through the discussion, the expertise of the senior author (I.D.) was involved to finally reach a uniform consensus. The initial search through the abovementioned databases resulted in identifying 1058 records, 322 of which were duplicates. Therefore, 736 titles were screened, and 670 were excluded because of inappropriate type or irrelevance. 66 abstracts were assessed, 48 of which were found to be unrelated to the topic. In the final search of the 18 full-text articles, six [28, 29] did not compare two different types of headaches directly to each other. More precisely, one study only compared primary headaches as a whole to healthy controls [28], one analysed inflammatory neurological diseases [29], one included patients with a traumatic brain injury without headache assessment [30], one distinguished, among groups, headache patients but without further differentiation [31], and finally, two studies analysed different types of headaches, however, did not compare them to each other but only to healthy controls [32, 33]. Therefore, 12 were chosen for inclusion.

Additionally, reference lists of the indicated articles were hand-screened for potential studies. Out of 754 references, 199 were identified as duplicates, and 453 were found outside the review topic. Thus, 102 studies were screened. One report was not retrieved, and 101 were assessed, resulting in nine more articles being selected [34–42]. Therefore, the final number of included studies in the systematic review was 21 (Fig. 1). All full texts were

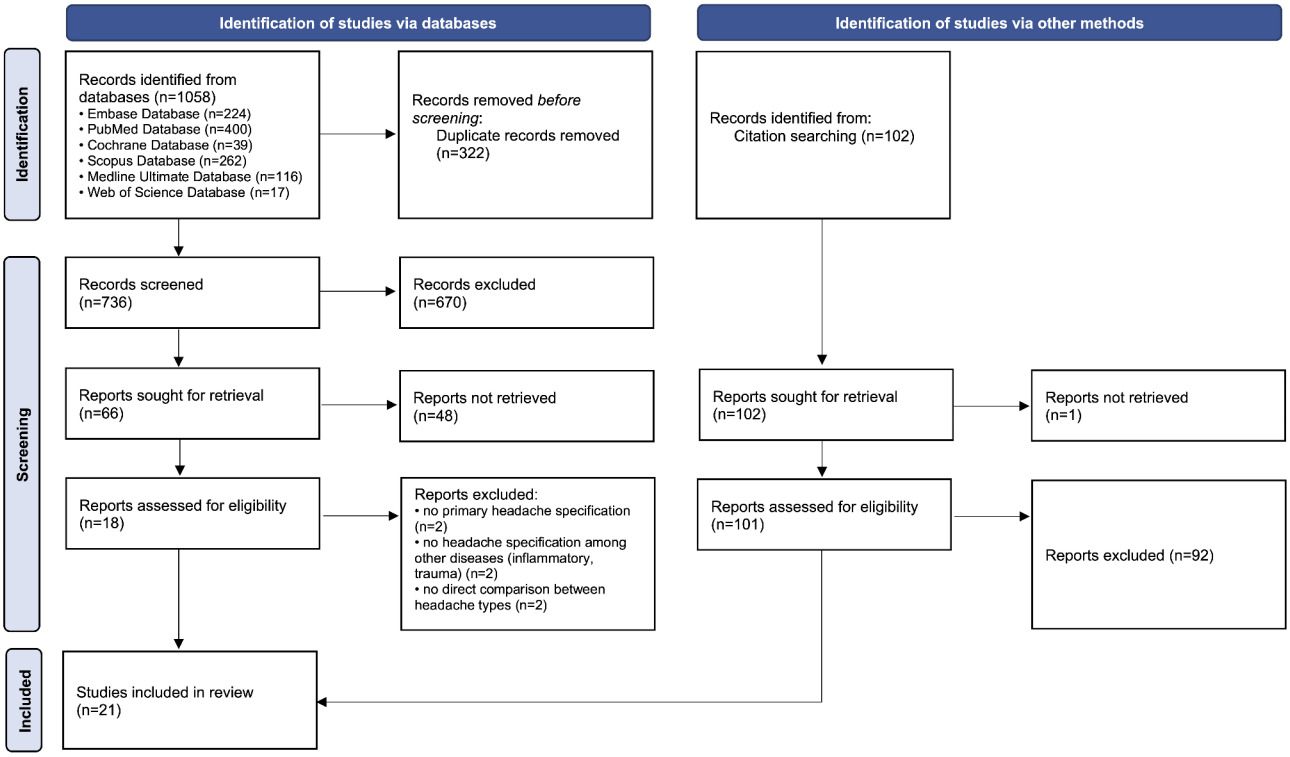

Fig. 1 A flowchart presenting a selection process according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA 2020) [27]. n, number of studies

available, and we did not find any unclear or inconsistent data in the analysed articles.

Data extraction and data synthesis

Relevant data were collected using established principles to maintain accuracy and consistency. Two independent reviewers (O.G. and W.Ł.) selected the essential study details, resolving any discrepancies through discussion and, if necessary, involving the senior author's expertise (I.D.) to reach a common consensus and decrease the risk of bias.

Data synthesis was performed narratively to identify overarching patterns. Established arrangements were followed to ensure the reliability and validity of the whole process. For each included study, the most appropriate methods of results presentation were implemented (percentages, mean values, accuracy, sensitivity, specificity).

Quality assessment

All studies included in the systematic review were assessed for risk of bias using the Prediction Model Risk of Bias Assessment Tool (PROBAST), a tool for assessing the risk of bias and the applicability of diagnostic and prognostic prediction model studies [43]. The risk of bias assessment was performed primarily by the first author (O.G.) and revised by two other authors (W.Ł., I.D). Discrepancies, if any, were resolved through the discussion, leading to a consistent consensus. There were no studies in which a consensus was not reached. Most of the studies have been assessed as having a low concern regarding applicability. However, the majority of them did not meet the criteria for the low risk of bias. Firstly, due to the lack of information about the sample size calculation with the reasonability of the number of participants with the outcome, the risk of bias was marked for some studies as unclear. Secondly, when the authors stated the limitation of a small sample size, the risk of bias had to be estimated as low. Therefore, while considering the systematic review findings, it should be remembered that the quality of some of the included studies may be decreased, mainly due to the limited number of patients included. The results of the PROBAST analysis, with indications of studies with lower and unclear risk of bias, are shown in Table 1.

Findings

Every study meeting the inclusion criteria has been analysed and included in the appropriate paragraph of the systematic review. Performing a comprehensive search through six databases allowed us to minimize the risk of omitting any valuable article. Strictly adhering to PRISMA 2020 guidelines ensures the reader that all essential methodological details have been applied. Studies selected through the database search covered

Table 1 The tabular presentation of prediction model risk of Bias assessment tool (PROBAST) results

Study	ROB				Applicability			Overall	
	Participants	Predictors	Outcome	Analysis	Participants	Predictors	Outcome	ROB	Applicability
Sarchielli et al. [34]	-	+	+	?	+	+	+	-	+
Leone et al. [35]	+	+	+	?	+	+	+	?	+
Forcelini et al. [36]	+	+	+	-	+	+	+	-	+
Giorgio et al. [37]	+	+	+	-	+	+	+	-	+
Chong et al. [38]	+	+	+	?	+	+	+	?	+
Schwedt et al. [39]	+	+	+	?	+	+	+	?	+
Dumkrieger et al. [40]	+	+	+	?	+	+	+	?	+
Nicolodi et al. [41]	+	?	+	-	+	+	+	-	+
Rossi et al. [42]	+	+	?	?	+	+	?	?	?
Mauskop et al. [44]	?	+	+	?	+	+	+	?	+
Ferrari et al. [45]	?	+	+	?	+	+	+	?	+
Cernuda-Molloron et al. [46]	+	+	+	?	+	+	+	?	+
Cernuda-Molloron et al. [47]	+	+	+	?	+	+	+	?	+
Carlsen et al. [48]	+	+	+	+	+	+	+	+	+
Fan et al. [49]	+	+	+	-	+	+	+	-	+
Yang et al. [50]	+	+	+	+	+	+	+	+	+
Zhang et al. [51]	+	+	+	-	+	+	+	-	+
Sollmann et al. [52]	+	+	+	-	+	+	+	-	+
Kazanci et al. [53]	+	+	+	?	+	+	+	?	+
Messina et al. [54]	+	+	+	-	+	+	+	-	+
Unay et al. [55]	+	+	+	?	+	+	+	?	+

ROB, risk of bias

+ indicates low ROB/low concern regarding applicability

- indicates high ROB/high concern regarding applicability

? indicates unclear ROB/unclear concern regarding applicability

four main types of biomarkers: blood biomarkers [34–36, 44–51], imaging biomarkers [37–40, 52–54], saliva biomarkers [34, 41], and evoked potential biomarkers [42, 55]. Most researchers conducted their analyses on the adult population; however, studies assessing the value of evoked potentials were performed only on the paediatric population [42, 55]. Also, one of the blood biomarker studies was conducted on children [49]. The most analysed headache was migraine in comparison to other types of primary headaches, including TTH [34, 35, 44, 45, 52, 53] or CH [37, 38, 46, 47, 54], or secondary headaches, such as medication overuse headaches (MOH) [36, 48] and post-traumatic headaches (PTH) [39, 40].

Blood biomarkers

Migraine and tension-type headaches differentiation

Biomarkers the most commonly assessed in headaches appeared to be blood biomarkers, with the comparison between migraine and TTH leading. Two out of four studies on this topic analysed the diagnostic role of magnesium (Mg) [34, 44]. Mauskop et al. [44] conducted a study in which researchers divided patients with chronic daily headaches into two groups: demonstrating and non-demonstrating migraine features; however, researchers did not distinguish between headache and headache-free patients at the time of sample collection. It appeared that ionized Mg levels were low in serum in 30.8% (8/26) of the former group in comparison to 4.5% (1/22) of the latter group. Also, more patients with daily chronic migraine compared to those with daily chronic TTH had high calcium/Mg ratios (60.1% (16/26) and 30.4% (8/22)). These results remain consistent with the study performed by Sarchielli et al. [34] in which Mg levels were compared between the following four groups: migraine with aura, migraine without aura, TTH, and healthy controls. No information about the presence of headache at the time of collection was included; however, participants were free from any drugs 20 days prior to blood collection. Patients with headaches presented significantly lower Mg levels than healthy individuals. Moreover, focusing on our review question, participants with both migraines with and without aura had further significantly decreased Mg concentration in comparison to the TTH group (mean values [mmol/l]: 0.72 ± 0.10, 0.81 ± 0.09, and 0.89 ± 0.09, respectively). Additionally, researchers assessed Mg levels in saliva, described below in the adequate paragraph.

Another research group, Leone et al. [35], compared leukocyte subsets in migraine patients without aura, chronic TTH patients, and healthy controls. Importantly, migraine patients were at least two days after the last attack, while TTH patients had mild or moderate

headaches during blood collection. From all the subsets, the statistical difference between migraine and TTH was presented for CD8 T-cells and CD4/CD8 ratio. The percentage of CD8 cells was similar in TTH patients and healthy individuals and significantly lower in migraineurs (mean values [%] ± standard deviation (SD): 30.5 ± 8.1, 30.1 ± 4.7, and 22.8 ± 6.2, respectively). The CD4/CD8 ratio was the highest for participants with migraine; however, it reached significance only in comparison to those with TTH (2.1 ± 0.8 and 1.3 ± 0.6, respectively). Finally, Ferrari et al. [45] measured platelets and platelet-poor plasma levels of methionine-enkephalin (MET) in drug-free patients with migraine (with and without aura) and TTH and compared them to the healthy control group. The results showed that plasma-MET concentrations were elevated in TTH patients in comparison to those with both types of migraine (with and without aura) and controls (mean values [10^{-21} g/platelet]: 68.1, 33.3, 28.3, and 39.6, respectively). On the contrary, platelets-MET levels were higher in migraineurs with and without aura than in TTH participants (34.3, 57.3, and 7.6, respectively).

Migraine and cluster headaches differentiation

Cluster headaches are the third most common type of primary headache that typically manifests with unilateral, orbital, or supraorbital pain with ipsilateral autonomic symptoms [3]. However, sometimes, the clinical presentation is more complex with features of both migraine and CH, and then the diagnosis may cause difficulties. Cernuda-Morrolón et al. [46] conducted a study in which episodic and chronic migraine patients were compared to those with episodic CH outside a symptomatic period and healthy individuals in terms of plasma CGRP concentration. Regarding migraine patients, no information about pain during collection was given. Results for chronic migraine patients differed from episodic migraine, CH, and control groups, being significantly higher (mean values ± SD [pg/ml]: 74.90 ± 28.29, 46.37 ± 15.21, 45.87 ± 12.32, and 33.74 ± 15.10, respectively). However, levels in episodic migraine and CH presented similar numbers; therefore, CGRP wasn't shown as a universal migraine vs. CH biomarker. In another study led by the same researchers [47], another peptide widely explored in migraine, the vasoactive intestinal peptide (VIP), was given a closer look. Similarly to CGRP, VIP appeared to be significantly higher in CM in comparison to episodic CH patients being outside a symptomatic period and healthy controls (mean values ± SD [pg/ml]:165.1 ± 125.4, 101.1 ± 78.6, and 88.6 ± 62.3, respectively). This peptide also did not present as a universal biomarker since the difference between EM (134.9 ± 80.4) and CM was not significant. Therefore, these two studies showed the role of CGRP and VIP in the differentiation of chronic but not episodic migraine with CH.

Migraine and medication overuse headache differentiation

Forcelini et al. [36] conducted a study comparing migraine patients to individuals with MOH, a type of secondary headache that may cause therapeutic problems for clinicians in their daily routine. Four groups were indicated: MOH, chronic migraine, episodic migraine with aura, and controls. All headache patients had their blood collected during their ictal phase, while those with migraine with aura also during the interictal phase. All chronic migraine and MOH patients, before final diagnosis, had a history of migraine without aura. Researchers measured lymphocyte count, which appeared to be significantly higher in patients with MOH compared to those with episodic migraine (mean value ± SD [/mm^3]: 2448.7 ± 775.8 vs. 1859.7 ± 564.7). However, the difference between any other pair was not significant. Carlsen et al. [48] also carried out a study on MOH patients (all with a pre-existing migraine or TTH diagnosis), comparing them to the controls consisting of individuals with episodic migraine patients and healthy participants. A higher neutrophile/lymphocyte ratio was demonstrated in the study group compared to the control group of episodic migraine and healthy individuals (mean ± SD: 2.49 ± 1.02, 1/86 ± 0.58, and 1.96 ± 0.63, respectively), suggesting an increased role of immune response in MOH.

Other headaches differentiation

Biomarkers in migraine patients were also assessed by Fan et al. [49] in comparison to non-migraine headaches in general and non-headache controls. Interestingly, this is the only study regarding blood biomarkers in headache differentiation conducted on the paediatric population. The mean age (±SD) of migraine children was 11.7±0.4 years, for the non-migraine group 9.6±0.7, and for the non-headache group 10.1±0.8. Noteworthy, this study did not distinguish the causes of non-migraine headaches, assuming they were one group regardless of the type. The analyses were performed with a distinction between attack-free patients and those during the attack. It was demonstrated that the migraine group presented higher levels of CGRP, both during and between attacks than the non-migraine and non-headache groups (mean value ± SD [pg/ml]: 291 ± 60, 240 ± 48, 51 ± 5, and 53 ± 6).

Yang et al. [50], in their study, addressed the up-to-date topic of the machine learning (ML) approach, specifically in the differential diagnosis of primary and secondary headaches. An ML-based predictive model evaluated ten standard parameters of a complete blood count test, 19 ratios of these ten parameters, and the patient's clinical and demographic features. The ten blood parameters were red blood cell count, platelet count, mean

corpuscular volume (MCV), white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, and haemoglobin. The final prediction model reached an accuracy of 0.7405 in differentiating primary and secondary headaches; sensitivity and specificity were 58% and 90%, respectively. Moreover, after Spearman's correlation matrix and feature weight analysis, the following parameters, white blood cell count, monocyte count, and neutrophil count, together with demographic data such as age and sex, were indicated as strongly correlated with headache type.

Zhang et al. [51] analysed patients with headache symptoms divided into two groups: patients diagnosed with spontaneous subarachnoid haemorrhage (SAH) and those with non-traumatic acute headaches due to other causes. Plasma levels of several potential markers were assessed, including prothrombin time (PT), international normalised ratio (INR), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen, and D-dimers. Importantly, since this study concerns the acute state, all measurements were taken before treatment or no later than 24 h after admission. No further information about the time of collection was given. Only two of them reached statistical significance: APTT decreased in the SAH group in comparison to non-SAH headaches (mean value ± SD [s]: 23.61 ± 5.06 and 27.17 ± 3.77, respectively), and D-dimers increased(mean value ± SD [mg/l]: 1.94 ± 2.24 and 0.26 ± 0.20, respectively). The area under the curve (AUC), illustrating the ability to classify data points accurately, was 0.721 for APTT and 0.886 for D-dimers; thus, D-dimers were shown to have a higher value than APTT in differentiating between primary and secondary headaches.

All studies regarding blood biomarkers in the differential diagnosis between headache types with additional information, such as the number of participants involved or methodology insights, have been summarised in Table 2.

Imaging biomarkers

Migraine and tension-type headache differentiation

In recent years, imaging biomarkers have gained much attention in the diagnosis of diverse diseases. In the field of neurology, most of these biomarkers relate to neuroimaging, especially magnetic resonance imaging (MRI) of the brain; however, some extend beyond the central nervous system. Such an example has been shown in a study conducted by Sollmann et al. [52], who performed a trapezius muscle MRI in patients suffering from both migraine and TTH and compared them to those with TTH only. The concept was based on the myofascial involvement in primary headache disorders and the relationship between headache and neck pain. It was shown that the group with migraine features presented the highest muscle T2 values (mean value ± SD [ms]: right side – 31.4 ± 1.2, left side – 31.2 ± 0.8) compared to both TTH-only (right side – 30.8 ± 1.1, left side – 30.9 ± 1.1) and control groups (right side – 30.0 ± 1.1, left side – 30.2 ± 1.1). Moreover, the results correlated significantly with the number of headache days and, unsurprisingly, with neck pain.

Kazancı et al. [53] analysed carotid intima-media thickness (CIMT) in migraineurs compared to TTH patients and healthy individuals. Furthermore, the migraine group was divided into ictal and interictal subgroups. However, no significant difference in CIMT was found between the groups (mean value ± SD [mm]: 0.46 ± 0.14, 0.43 ± 0.15, 0.45 ± 0.10, 0.50 ± 0.12, for TTH, migraine during remission, migraine attack, and controls, respectively).

Migraine and cluster headaches differentiation

Several recent studies focused on MRI changes between migraine and CH patients. Messina et al. [54] compared both headache groups to healthy controls regarding functional and structural MRI data and clinical presentation, applying multiple comparison corrections. All participants were in the headache-free phase. Moreover, CH patients had to stay in the "out-of-bout" phase to be included. The accuracy of the MRI classifier for distinguishing between migraine and CH groups was estimated at 78%; however, MRI-clinical combined classification reached an accuracy of 99%. Notably, the most discriminative feature here was the left thalamic network. More precisely, CH patients presented lower functional interaction between the left thalamus and cortical areas involved in the mediation of interoception and sensory integration than migraineurs and healthy controls. Therefore, the role of these areas should be carefully analysed in both types of headaches. Giorgio et al. [37] also analysed MRI data in three groups: migraine, episodic CH, and control. Headache patients were in their attack-free period. It was demonstrated that patients with CH had lower grey matter (GM) volume in frontal regions compared to migraineurs and healthy individuals. Moreover, decreased GM volume was also observed in CH patients in the lateral occipital area; however, this was only in comparison to migraine patients. More precisely, CH patients presented with higher functional connectivity than migraineurs in the working memory network (mean value ± SD: 20.16 ± 13.94 and 2.15 ± 8, respectively) and in the executive control network (21.5 ± 13.12 and 5.18 ± 5.27, respectively). Importantly, significance levels were corrected for multiple comparisons.

Chong et al. [38] compared T1-weighted MRI images in migraine and episodic CH patients, both in the interictal period, with healthy individuals. Each group presented a significant positive correlation when comparing right and left hypothalamic region volumes with cortical thickness

Table 2 A summary of studies covering blood biomarkers in headaches' differentiation

Ref.	Year	Population	Comparison	Biomarker	Results	Methodology
Mauskop et al. [44]	1994	26 pts with daily MIG headache	21 pts with daily TTH	Mg	↑ serum ionized Mg in 30.8% MIG pts compared to 4.5% of TTH ↑ ionized Ca/Mg ratio in 61.5% MIG pts compared to 36.4% of TTH	ionized Mg with ion selective electrodes total serum Mg by atomic absorption spectroscopy ratios calculated
Sarchielli et al. [34]	1992	41 MO pts 29 MA pts 30 TTH pts	40 HC	Mg	↓ Mg in MA and MO than in TTH ↓ Mg in MA, MO, and TTH than in HC	serum Mg by atomic absorption spectroscopy
Leone et al. [35]	1994	12 MO pts 12 chronic TTH pts	12 HC	leukocytes subsets	↑ B-lymphocytes level in TTH than HC ↓ CD8 T-cells level in MIG than TTH and HC ↑ CD4/CD8 in MIG than TTH	cells counted by Coulter Counter ratios calculated
Ferrari et al. [45]	1990	10 MA pts 21 MO pts 9 TTH pts	9 HC	MET in PLT and PLT-poor plasma MET	↑ PLT MET in MO than TTH and HC ↑ plasma-MET in TTH than in MIG ↑ MET ratio in MIG than in TTH and HC ↑ PLT and plasma-MET during MIG attacks than interictally	PLT counted electronically MET determined by RIA
Cernuda-Morrolón et al. [46]	2013	103 CM female pts 43 EM female pts 14 CH pts	31 female HC	CGRP	↑ CGRP in CM than in EM, CH, and HC	CGRP by ELISA absorption levels with a spectrophotometer
Cernuda-Morrolón et al. [47]	2015	119 CM female pts 51 EM female pts 18 CH pts	33 female HC	VIP	↑ VIP in CM than in CH and HC ↑ VIP in EM than in HC NSD between EM and CM (numerically ↑ VIP in CM)	VIP by ELISA absorption levels with a spectrophotometer
Forcelini et al. [36]	2011	17 MOH pts	17 MO/CM pts	leukocytes subsets, haemoglobin, haematocrit	↑ lymphocyte count in MOH pts than in MO pts NSD between any other groups	WBC counted, haemoglobin, haematocrit in Cell Dyn 3000
Carlsen et al. [48]	2023	120 MOH pts	29 EM pts 28 HC	leukocytes subsets	↑ neutrophile/lymphocyte ratio in MOH than in control group ↑ reduction of headache days/month associated with ↓ neutrophile/lymphocyte ratio	by DNA extraction, bisulfite-convertion and analysis
Fan et al. [49]	2019	68 MIG paediatric pts	30 non-MIG headache paediatric pts 22 healthy children	CGRP	↑ CGRP in MIG pts than in non-MIG pts and HC (ictally and interictally)	CGRP by ELISA
Yang et al. [50]	2023	ML predictive model based on 121,241 pts	-	blood parameters and their ratios, patient's features	accuracy of 0.7405 sensitivity: 90%, specificity: 58%, false negative rate: 10%, false positive rate: 42%	data from the UK Clinical Practice Research Datalink prediction model developed by using two ML-based approaches data normalization and optimization by min–max scaling
Zhang et al. [51]	2019	46 spontaneous SAH pts	68 headache pts due to other causes	PT, INR, APTT, TT, fibrynogen and DD	NSD in PT, INR, TT, fibrynogen APTT accuracy: 0.721; DD accuracy: 0.886 cut-off values: 25.2s (sensitivity: 73.53%, specificity: 60.87%) for APTT; 0.31 mg/L (sensitivity: 83.82%, specificity: 84.7%) for DD	data gathered from medical records from the Fourth Affiliated Hospital Zhejiang University School of Medicine

↑, increase; ↓, decrease; APTT, activated partial thromboplastin time; Ca, calcium; CGRP, calcitonin gene-related peptide; CH, cluster headache; CM, chronic migraine; DD, D-dimers; DNA, Deoxyribonucleic Acid; ELISA, enzyme-linked immunosorbent assay; EM, episodic migraine; HC, healthy controls; INR, international normalised ratio; MA, migraine with aura; MET, methionine-enkephalin; MET ratio, platelets to plasma MET; Mg, magnesium; MIG, migraine; ML, machine learning; MO, migraine without aura; MOH, medication overuse headache; NSD, no significant difference; PLT, platelets; pts, patients; PT, prothrombin time; Ref, reference; RIA, radioimmunoassay; SAH, subarachnoid haemorrhage; TT, thrombin time; TTH, tension-type headache; VIP, vasoactive intestinal peptide

measurements. Covariance patterns in the post-hoc analysis were significantly different in migraine and CH compared to healthy controls. Both headache groups presented a weaker structural covariance of hypothalamic region volume with cortical thickness of the frontal and temporal lobes. The explanation for this observation may lie in the abnormal functioning of the pain control circuity. However, the presented method does not distinguish between headache types.

Migraine and post-traumatic headache differentiation

Finally, two other studies covered the MRI differences between migraine and PTH. Schwedt et al. [39] compared regional volumes, cortical thickness, surface area, and curvature measurements between migraineurs and patients affected by persistent PTH after mild brain injury. Moreover, patients with a history of headache diagnosis prior to PTH development were excluded to avoid bias. The following structures differed significantly between PTH and migraine patients: the right lateral orbitofrontal lobe (mean value ± SD [mm]: 0.0287 ± 0.0029 and 0.0301 ± 0.0005, respectively), left caudal middle frontal lobe (2.3787 ± 0.1648 and 2.5027 ± 0.1245, respectively), left superior frontal lobe (2.5637 ± 0.0339 and 2.6788 ± 0.1909, respectively), left praecuneus (2.2948 ± 0.0898 and 2.3746 ± 0.0460, respectively), and right supramarginal gyrus (2.4909 ± 0.0651 and 2.5916 ± 0.2086, respectively). More precisely, thickness, area, or volume measurements in patients with PTH were less than those within the migraine group. Researchers suggest looking for an explanation of these observations on the pathophysiological basis of both headaches.

Dumkrieger et al. [40] enrolled in their study patients with migraine, persistent post-traumatic headaches, and healthy participants. Headache patients were enrolled regardless of the intensity of pain at the time of imaging. Seventeen region pairs showed significant differences in static functional connectivity between the analysed two types of headaches. Among them, the following areas of interest were found: primary somatosensory, secondary somatosensory, posterior insula, hypothalamus, anterior cingulate, middle cingulate, temporal pole, supramarginal gyrus, superior parietal, middle occipital, lingual gyrus, pulvinar, praecuneus, cuneus, somatomotor, ventromedial prefrontal cortex, and dorsolateral prefrontal cortex. On the other hand, significant differences were observed between the same two groups in dynamic functional connectivity in ten region pairs, which included areas as follows: secondary somatosensory, hypothalamus, middle cingulate, temporal pole, supramarginal gyrus, superior parietal, lingual gyrus, somatomotor, precentral, posterior cingulate, middle frontal, fusiform gyrus, parietal-occipital, and amygdala. Additionally, although the first eight regions overlap with the static group, some are unique for each type of connectivity. These results showed functional differences in areas responsible for pain development. The significance thresholds were corrected for multiple comparisons. Noteworthy, if not mentioned while describing the results, the multiple comparisons correction was not performed.

All studies regarding imaging biomarkers in the differential diagnosis of headache types with additional information, such as the number of participants involved or methodology insights, have been summarised in Table 3.

Saliva biomarkers

Undoubtedly, the topic of biomarkers that could be found in saliva in headache differential diagnosis is less explored than the topic of blood and imaging biomarkers. Nevertheless, two studies analysed concentrations of particular molecules in saliva. However, both were conducted over thirty years ago, and novel research is lacking. Nicolodi et al. [41] measured levels of CGRP, substance P (SP), and VIP in patients suffering from migraine with aura and episodic CH and compared them to healthy controls. Interestingly, VIP levels during interictal and ictal periods acted differently in migraine and CH patients. A significant decrease during the migraine attack was observed in the former group (mean value ± SD [pmol/1]: from 138.8 ± 28.7 to 23.4 ± 8.9), while in the latter, VIP increased during the CH attack (from 114.6 ± 17.9 to 174.8 ± 9.5). Substance P and CGRP levels increased significantly in the ictal period in both migraine and CH patients. However, in this study, no direct comparison between basal levels of any of the substances in migraineurs and CH sufferers was performed.

Also, in the already mentioned study by Sarchielli et al. [34], researchers analysed Mg levels not only in blood but also in saliva. Three groups were compared: migraine, TTH, and healthy controls. Similarly to the blood results, salivary Mg concentration appeared lower in migraines with and without aura than in TTH, while healthy individuals presented the highest levels of all the groups (mean value ± SD [μmol/ml]: 0.11 ± 0.01, 0.12 ± 0.02, 0.14 ± 0.01, and 0.17 ± 0.02, respectively).

All studies regarding saliva biomarkers in the differential diagnosis between headache types with additional information, such as the number of participants involved or methodology insights, have been summarised in Table 4.

Evoked potentials

Finally, evoked potentials were analysed, comparing migraine and TTH in two studies; however, both were performed on the paediatric population. Unay et al. [55] conducted a study evaluating visual evoked potentials (VEP) and brainstem auditory evoked potentials

Table 3 A summary of studies covering imaging biomarkers in headaches' differentiation

Ref.	Year	Population	Comparison	Imaging type	Results
Sollmann et al. [52]	2023	12 TTH + MIG pts 16 TTH only pts	22 HC	3T MRI of neck and shoulder region	↑ trapezius muscle T2 values in TTH + MIG pts than in TTH pts and HC ↑ muscle T2 values associated with ↑ headache days and neck pain
Kazancı et al. [53]	2019	23 MIG pts ictally 20 MIG pts interictally 20 TTH pts	21 HC	carotid intima media thickness by USG	NSD between any groups
Messina et al. [54]	2023	20 MIG pts 20 CH pts	15 HC	brain 3T MRI (structural and functional data)	accuracy of CH and MIG differentiation of 78% (99% when combined with clinical data) left thalamic network as the most discriminative feature
Giorgio et al. [37]	2020	13 MIG pts 12 CH pts	13 HC	brain 3T MRI (T1- and T2- weighted)	↓ grey matter volumes of frontal regions in CH than MIG and HC and of occipital regions compared to MIG ↑ FC of working memory and executive control networks in CH than MIG and HC and of cerebellar and auditory language comprehension networks than MIG
Chong et al. [38]	2020	19 MIG pts 18 CH pts	22 HC	brain 3T MRI (T1-weighted)	+ correlation for each group between right and left hypothalamic region volumes with cortical thickness ↓ structural covariance of hypothalamic region volume with frontal and temporal cortical thickness in MIG and CH than HC
Schewdt et al. [39]	2017	28 MIG pts 28 PPTH pts	28 HC	brain 3T MRI (T1-weighted)	differences between MIG and PPTH of structure of R lateral orbitofrontal, L caudal middle frontal, and L superior frontal lobes and L praecuneus and R supramarginal gyrus differences between PPTH and HC in structure of R lateral orbitofrontal lobe, R supramarginal gyrus, and L superior frontal lobe NSD between MIG and HC in those structures
Dumkrieger et al. [40]	2019	33 MIG pts 44 PPTH pts	36 HC	brain 3T MRI (T1- and T2-weighted)	differences between MIG and PPTH in static FC in 17 regions pairs differences between MIG and PPTH in dynamic FC in 10 regions pairs

↑, increase; ↓, decrease; +, positive; CH, cluster headache; FC, functional connectivity; HC, healthy controls; L, left; MIG, migraine; MRI, magnetic resonance imaging; NSD, no significant difference; PPTH, persistent post-traumatic headache; pts, patients; R, right; Ref, reference; T, Tesla; TTH, tension-type headache; USG, ultrasonography

Table 4 A summary of studies covering saliva biomarkers in headaches' differentiation

Ref.	Year	Population	Comparison	Biomarker	Results	Methodology
Nicolodi et al. [41]	1990	15 MO pts 15 CH pts (5 interictally, 10 ictally)	16 HC for MIG 28 HC for CH	CGRP, SP, VIP	VIP during attack ↑ in CH and ↓ in MO CGRP and SP during attack ↑ in CH and MO ↑ CGRP and SP in CH interictally than in HC ↓ CGRP and SP in MO interictally than in HC ↑ VIP and SP in CH during attack on symptomatic side and stable levels on non-symptomatic side	CGRP, SP, VIP by RIA
Sarchielli et al. [34]	1992	41 MO pts 29 MA pts 30 TTH pts	40 HC	Mg	↓ Mg in MA and MO than in TTH ↓ Mg in MA, MO, and TTH than in HC	saliva Mg by atomic absorption spectroscopy

↑, increase; ↓, decrease; CGRP, calcitonin gene-related peptide; CH, cluster headache; HC, healthy controls; MA, migraine without aura; Mg, magnesium; MO, migraine without aura; pts, patients; Ref, reference; RIA, radioimmunoassay; SP, substance P; TTH, tension-type headache; VIP, vasoactive intestinal peptide

(BAEP) in children with headaches. All patients were in their headache-free phase for at least a week and did not take prophylactic drugs. The mean age of the children was 10.4 years (range: 6–13 years). The parameters of VEP, precisely P100 latency (mean value ± SD [ms]: 103.94 ± 5.09, 101.03 ± 4.19, 100.62 ± 3.67) and amplitude (11.9 ± 1.5, 10.8 ± 1.2, 10.3 ± 0.9 [μV]), appeared significantly higher in migraine patients compared to those with TTH and healthy controls, respectively. However, the differences in BAEP response did not reach significance in any performed comparison. Rossi et al. [42] assessed VEP in children with different types of migraine, TTH, and healthy individuals (the mean age of each group was 9 years, minimum 1-year-old). The examination was performed at least 48 h after the last headache attack. However, contrary to the previously described study, no differences were found between VEP while comparing all three groups. Those discrepancies between studies show the need for further research on the topic.

All studies regarding evoked potential biomarkers in the differential diagnosis of headache types with additional information, such as the number of participants involved, have been summarised in Table 5.

Conclusions and future perspectives

A comprehensive analysis of selected studies regarding biomarkers in headache differential diagnosis leads to several conclusions. Undoubtedly, all of them should be taken carefully since, to our knowledge, this is the first systematic review summarizing biomarkers strictly in headaches differentiation between the types, and no comparison with other works can be conducted. The highest number of studies included in the review have been conducted on the role of blood biomarkers. However, four of those studies were conducted in the nineties [34, 35, 44, 45], while only two were in the past five years [48, 49]. On the contrary, fewer articles focused on imaging biomarkers in general, however, with four being conducted in the past five years [37, 38, 52, 53] and the oldest study published only 8 years ago [39]. The growing interest in imaging biomarkers suggests they may play an increasing role in migraine differential diagnosis, though further validation is needed. The exact location of those biomarkers is presented in Fig. 2. Only two studies of each biomarker type were conducted on saliva [34, 41] and evoked potential biomarkers [42, 55], showing a lack of evidence in this area. Moreover, those studies were also conducted decades ago, with the newest published over 15 years ago. The question can be raised whether there are no studies conducted on the topic or no biomarkers that can be promising. Additionally, the accuracy, AUC, sensitivity, or specificity of the biomarkers analysed in each study was summarised in Table 6. However, in only four out of 21 studies, at least one of these parameters was mentioned, which causes a significant limitation and suggests caution when drawing conclusions. Finally, none of the selected studies analysed cerebrospinal fluid biomarkers. This, however, would not significantly influence daily practice, as conducting lumbar puncture in primary headache patients is rare [56].

The literature on biomarkers distinguishing headache types is generally very limited. However, the number of studies is relatively high in terms of differentiating one type of primary headache from healthy controls. There are high-value systematic reviews regarding biomarkers in migraine [20, 57], CH [22], or primary headache disorders in general [21]. Besides imaging, genetic, and provocation biomarkers [20], molecules such as hormones, prolactin, catecholamines, melatonin [20, 22], or small non-coding ribonucleic acids (RNA) [57] have been proposed to play a role in headache management. Moreover, the role of cytokines in headaches is drawing growing attention, with some of them being proven to increase in migraine compared to healthy controls [58]. Inflammatory factors, such as interleukin 6 (IL-6), IL-8, IL-1β, and tumour necrotic factor alpha (TNF-α) putatively play a role in migraine pathogenesis, being additional molecules that can be considered future biomarkers, either diagnostic or prognostic [58], showing that the problem of lack of literature is relatively isolated to the very narrow area of differential diagnosis between headache types. Also, the vast majority of the already limited number of studies focus on migraine headaches and compare them to other types. Research analysing at least two headache types different than migraine is very rare [51], indicating a high need for further studies. Finally, only three out of 21 studies focused on the paediatric population [42, 49, 55], while children's headaches are considered no less important problem globally [59].

Another vital issue worth raising here is the difference between the implication of biomarkers in daily routine and primary and secondary headache disorders. While there are barely any validated biomarkers in the field for the first one, some secondary headaches have biomarkers included in their diagnostic pathways [3]. This presumably relates to the well-known pathogenesis of secondary headache disorders, such as inflammation in headaches attributed to giant cell arteritis and headaches attributed to infection or mechanic traumas in post-traumatic headaches [3]. Thus, inflammatory markers [60] or imaging alterations [3] are promising in each type. Moreover, imaging biomarkers are also crucial in some types of secondary headaches that are not preceded by any specific factor, such as idiopathic SAH [61]. In contrast, the molecular bases of primary headaches are not yet completely understood and discovered [62], which undoubtedly hinders further attainments in the field of primary headaches' biomarkers.

Diagnosing headaches can sometimes be challenging, with overlapping symptoms and uncommon manifestations [4, 10]. Underdiagnosis of primary headaches,

Table 5 A summary of studies covering evoked potential biomarkers in headaches' differentiation

Ref.	Year	Population	Comparison	Biomarker	Results
Unay et al. [55]	2008	37 MIG paediatric pts 35 TTH paediatric pts	40 healthy children	BAEP, VEP	↑ P100 latency and amplitude (VEP) in MIG than TTH or HC NSD in P100 latency and amplitude between TTH and HC
Rossi et al. [42]	1996	71 MIG or TTH paediatric pts	19 healthy children	VEP	NSD in P100 latency (VEP) between MIG, TTH and HC

↑, increase; BAEP, brainstem auditory evoked potentials; MIG, migraine; pts, patients; Ref, reference; SP, substance P; TTH, tension-type headache; VEP, visual evoked potentials

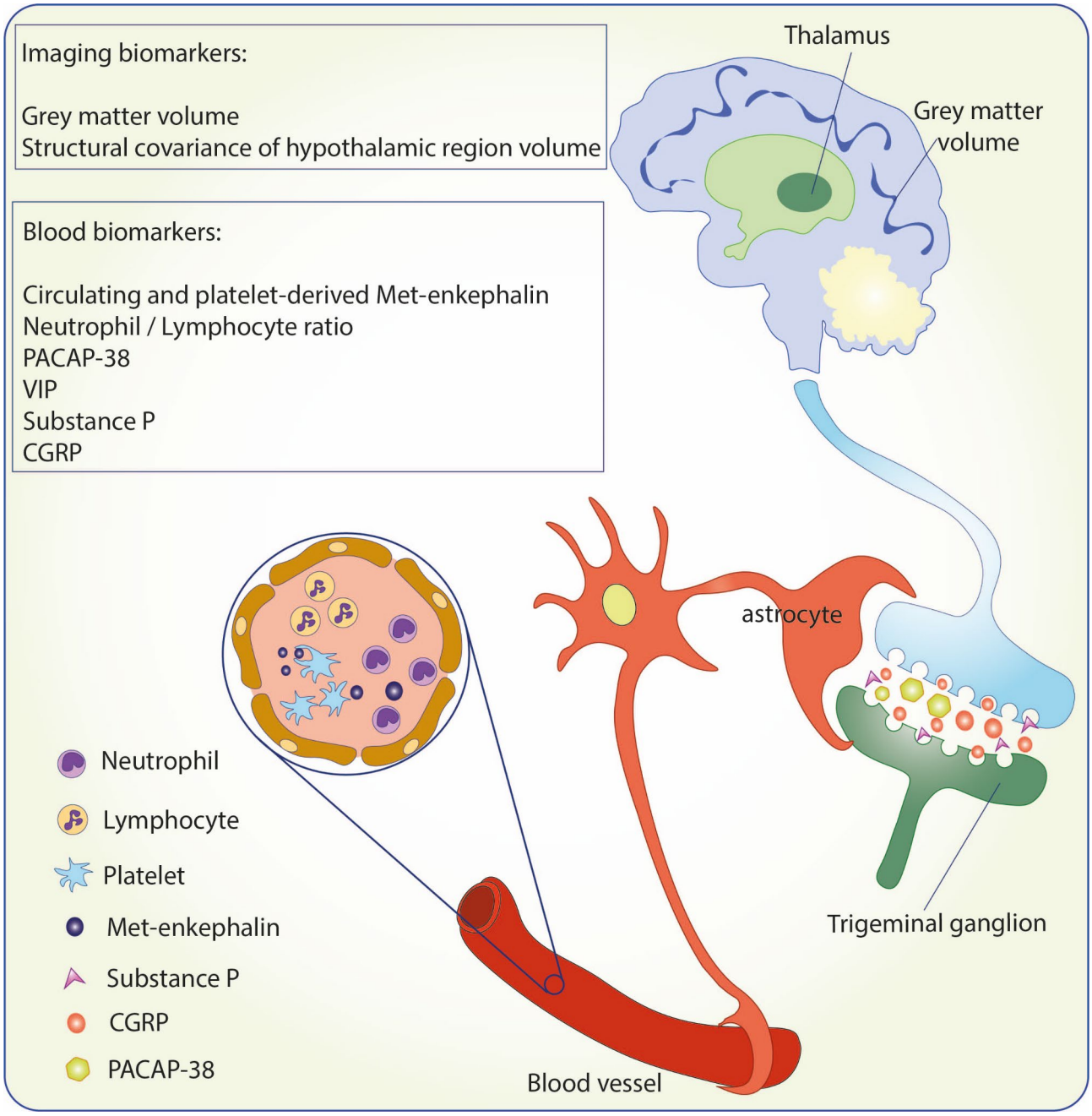

Fig. 2 A graphical presentation of locations where some headache biomarkers can be found. CGRP, calcitonin gene-related peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide; The Adobe Illustrator Program licensed by C. E. was used to prepare the figure

mainly migraine, is a rising issue worldwide since migraine and headaches, in general, are important causes of years lived with disability [11–13]. Therefore, improving the diagnostic pathway by applying specific biomarker measurements could be a promising solution, especially in atypical cases. Notably, a higher rate of accurate diagnoses is closely related to better management and more appropriate treatment, which has significant advantages for patients [63, 64].

Finally, an important point, however, beyond the direct topic of this review, is headache pathogenesis. Identifying specific biomarkers that differentiate between headache types and analysing their role in each type may lead to a better understanding of the molecular bases underlying primary headache development [20]. For instance, studies showing increased levels of inflammatory factors in migraineurs suggest inflammation involvement in migraine pathogenesis [35]. A better understanding is crucial for discovering targeted therapies and applying

Table 6 The accuracy, AUC, sensitivity, and specificity of biomarkers used in each of the mentioned studies, if it mentioned at least one of the parameters. The red background is used for blood biomarkers, and the grey for imaging biomarkers

Study	Biomarker	Headache types	Accuracy	AUC	Sensitivity	Specificity
Yang et al. [50]	blood parameters and their ratios, patient's features	primary vs. secondary headaches	0.7405 (balanced accuracy)	0.8519	58%	90%
Zhang et al. [51]	APTT (25.2 s.)	spontaneous SAH vs. acute non-traumatic headache	not given	0.721	60.87%	73.53%
	DD (0.31 mg/l)		not given	0.886	84.78%	83.82%
Sollmann et al. [52]	3T MRI of neck and shoulder region	TTH vs. TTH + migraine	not given	0.69	not given	not given
Messina et al. [54]	brain 3T MRI (structural and functional data)	migraine vs. CH	99%	not given	95%	100%

APTT, activated partial thromboplastin time; AUC, area under the curve; CH, cluster headaches; DD, D-dimers; MRI, magnetic imaging resonance; SAH, subarachnoid haemorrhage; TTH, tension type headache; vs., versus

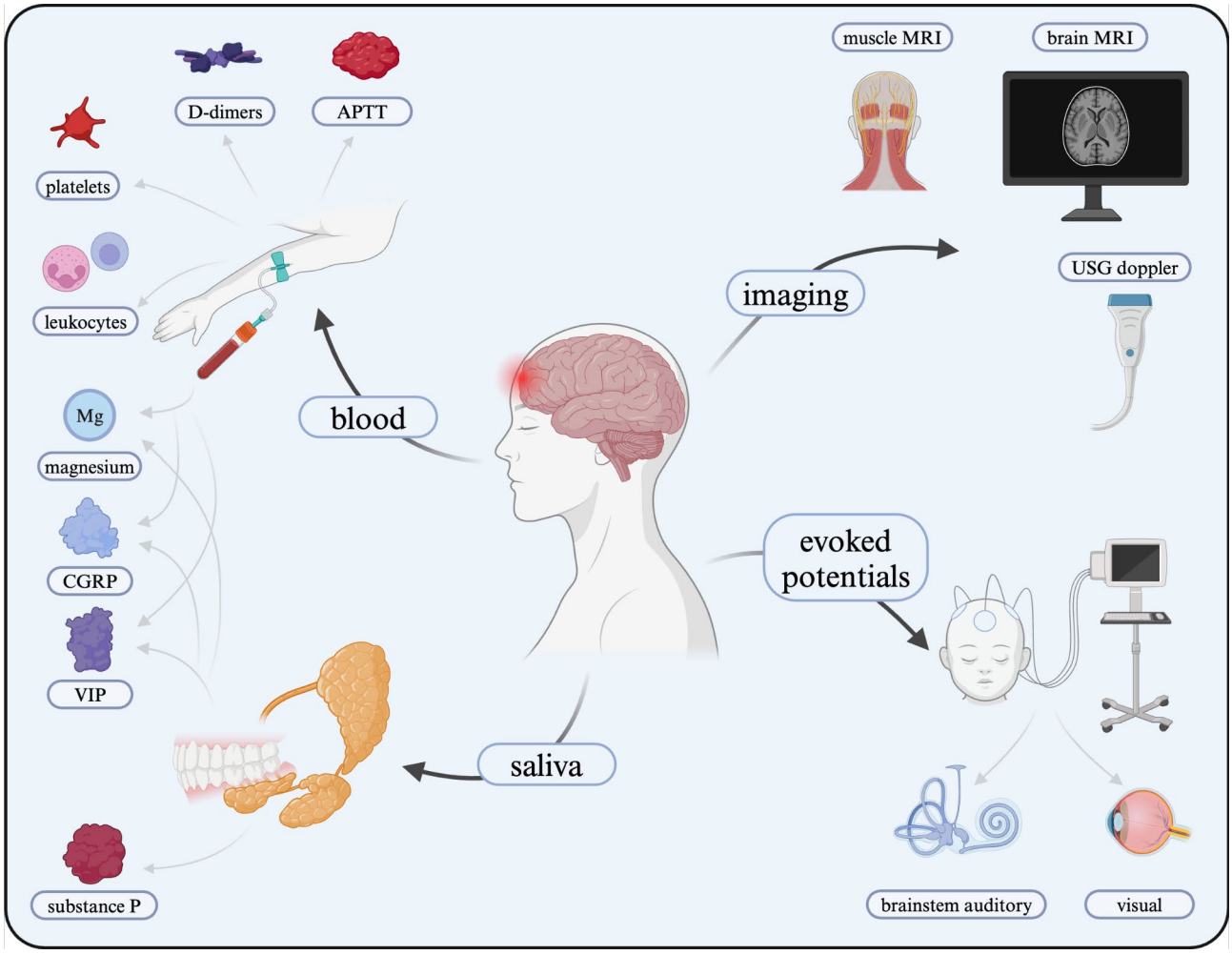

Fig. 3 A graphical summary of all biomarker types studied in headache differential diagnosis. APTT, activated partial thromboplastin time; CGRP, calcitonin gene-related peptide; MRI, magnetic resonance imaging; USG, ultrasonography; VIP, vasoactive intestinal peptide; The BioRender Program licenced by O.G. was used to prepare the figure

causal treatment [20]. Individualised management can help achieve better outcomes and a higher rate of positive responses to applied medications, resulting in a better quality of life for headache patients [65]. Figure 3 summarizes the biomarker types analysed in the included studies.

Although biomarkers can be considered an opportunity for the vast facilitation of clinical decision-making, some barriers to their application to daily routines should

be discussed. Costs and accessibility are the clear disadvantages in comparison to diagnosis based on clinical criteria, especially when it comes to the expenses on imaging biomarkers [66]. The second important issue is biological differences, including different populations and circadian or seasonal changes [67, 68]. Therefore, the use of specific biomarkers may be limited to particular populations, or at least, different thresholds should be applied for each of them. Similarly, the time of sample collection, or even a season, can sometimes be crucial in interpreting the results and should not be forgotten [69]. Additionally, technical issues, such as the reliability or stability of biomarkers over time using different diagnostic tests, can arise more often than during the currently approved diagnostic process [70]. Even if a specific biomarker is effective in differential diagnosis, its everyday use could be limited due to aspects such as its short half-life or lack of reproducibility of the results [70]. Finally, in some acute states, such as SAH analysed in one of the referenced studies [51], performing biomarker measurements, although potentially effective, could be of less value than fast clinical assessment since time is crucial in such situations [71, 72]. Therefore, performing unnecessary tests before making the decision regarding the management and treatment could be considered malpractice [73]. Many studies have shown that biomarkers can facilitate differential diagnosis of headaches, and this systematic review reveals the exact directions and methods. However, further investigations conducted on more significant samples are needed, which should focus primarily on imaging biomarkers, as we found them to be the most promising ones, and blood biomarkers, whose undeniable advantage is their ability to be performed in daily clinical routines.

Limitations

This systematic review has some limitations. A simple search strategy was implemented in the review; therefore, there is a risk that some research was omitted. Several studies covered in the paper have a high risk of bias, mainly due to a limited number of enrolled patients. Therefore, all the conclusions should be drawn carefully. Also, a different methodology was applied in the included studies, even those assessing the same biomarkers, which could decrease the value of synthesized data. Nine of the described studies were conducted over ten years ago, which causes a risk of outdated data. Moreover, due to the wide variety of publication dates, headache diagnostic criteria could differ among articles from different years, increasing the information heterogeneity. Specifically, in seven articles, ICHD-1 criteria were applied [34, 35, 41, 42, 44, 45, 55], in three articles, ICHD-2 [36, 46, 47], and in the following nine, ICHD-3 [37–40, 48, 49, 52–54]. In two articles, data were collected from medical records based on the headache diagnosis made by a professional [50, 51], which is also an apparent limitation. The information about being in a headache's ictal or interictal phase was not always given. The term "non-migraine headache" used in one study [49] is unspecific and can be considered a limitation since the authors do not mention its precise definition. Not all studies' authors analysing CH have clarified the headache type (chronic/episodic, in remission/active). We considered only studies written in English or Polish, potentially limiting the number of appropriate studies included. This limitation may lead to bias in including studies performed on specific populations using particular languages; therefore, articles from countries where other languages are the most acknowledged could be omitted. Also, one study was not retrieved during the selection process, which could lead to not including an appropriate article. Finally, a potential limitation of our study is missing data in some included studies. While we recognize this issue, we relied on the available data to draw conclusions and attempted to mitigate its impact through careful study selection and analysis.

Abbreviations

APTT	Activated partial thromboplastin time
AUC	Area under the curve
BAEP	Brainstem auditory evoked potentials
CGRP	Calcitonin gene-related peptide
CH	Cluster headache
CIMT	Carotid intima-media thickness
CSF	Cerebrospinal fluid
GM	Grey matter
ICHD-3	International Classification of Headache Disorders, 3rd edition
INR	International normalised ratio
MCV	Mean corpuscular volume
Mg	Magnesium
ML	Machine learning
MOH	Medication overuse headaches
MRI	Magnetic resonance imaging
PRISMA	Preferred Research Items for Systematic Reviews and Meta-analyses
PROBAST	Prediction Model Risk of Bias Assessment Tool
PT	Prothrombin time
PTH	Post-traumatic headache
SAH	Subarachnoid haemorrhage
SP	Substance P
TT	Thrombin time
TTH	Tension-type headache
VEP	Visual evoked potentials
VIP	Vasoactive intestinal peptide

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